KINETICS OF ACTIN–MYOSIN BINDING

II. Two-variable Model and Actin Gelation

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ABSTRACT We consider a model of actin–myosin interaction in which the sites belonging to each seven-site regulated actin unit are subdivided into two classes, "internal" and "external." The time evolution of each class of sites is considered separately, leading to a pair of coupled differential equations that may be integrated numerically. We also consider the critical sol-gel transition point for actin filaments crosslinked by two-headed heavy meromyosin (HMM). The possibility of new types of chemical oscillation and pattern formation arising from periodic sol-gel transitions is discussed.

INTRODUCTION

In the previous paper (Klonowski and Epstein, 1986, hereinafter referred to as Part I) we introduced a simple one-variable model for the binding of myosin subfragment 1 (S1) to regulated actin. The model treats all actin sites as equally likely to be occupied (Part I, Eq. 13). Since the model allows for the intersite cooperativity to take different values between sites within a single unit and between sites at the ends of different adjacent units, the above assumption is clearly an oversimplification. While calculations with the one-variable model do give the qualitative features found experimentally (Trybus and Taylor, 1980) and with a more elaborate 16-variable model (Balazs and Epstein, 1983), it seems desirable to extend the simple model at least to recognize the difference between sites internal to and at the ends of actin units. We show here how to make that extension and give examples to show that the resulting two-variable model does indeed give good agreement with experiment.

The simplicity of the class of models considered makes it relatively easy to extend them to other types of binding. We treat the problem of binding the two-headed subfragment heavy meromyosin (HMM) with emphasis on crosslinking and the concomitant possibillity of sol-gel transitions. We derive an equation for the sol-gel transition point and suggest the possibility of a new type of chemical instability generated by sol-gel transitions.

A TWO-VARIABLE MODEL

The underlying seven-site periodicity of the model lattice (Balazs and Epstein, 1983) is suggested by structural studies (McLachlan and Stewart, 1976) and is illustrated in Fig. 1. This structure suggests a natural division of actin sites k into two classes, "internal" ($k = 2, 3, 4, 5, 6$) denoted by the subscript u, and "external" ($k = 1, 7$) denoted by the subscript z. The index r will be used to denote reacted (bound) actin sites, while the index f will refer to free sites and O will denote total numbers of sites.

Letting A stand for the number of sites of each class, we have

$$A_{iu} + A_{iz} = A_i \quad i = r, f, O \tag{1}$$

$$A_{rj} + A_{fj} = A_{Oj} \quad j = u, z. \tag{2}$$

We define

$$\theta_j = A_{rj}/A_{Oj} \quad j = u, z. \tag{3}$$

Since all regulated actin units are taken to be equivalent, we have

$$A_{Ou}/A_O = 5/7 \tag{4}$$

$$A_{Oz}/A_O = 2/7. \tag{5}$$

Combining Eqs. 3–5, we define the total degree of association (cf. Part I, Eq. 3) as

$$\theta = (5/7)\,\theta_u + (2/7)\,\theta_z \tag{6}$$

To calculate θ_u and θ_z we proceed by a technique analogous to that used in Part I, but instead of averaging over all actin sites together, we average separately over the five internal sites in each unit for θ_u and over the two external sites for θ_z. Instead of setting all the probabilities of occupation p_k to θ, we have (cf. Part I, Eq. 13)

$$p_k = \theta_u \quad k = 2, 3, 4, 5, 6 \tag{7a}$$

$$p_k = \theta_z \quad k = 1, 7. \tag{7b}$$

We obtain

$$d\theta_u/dt = M_O(1 - \gamma\theta)\,(1 - \theta_u)k_f e_{fu} - \theta_u k_r e_{ru} \tag{8a}$$

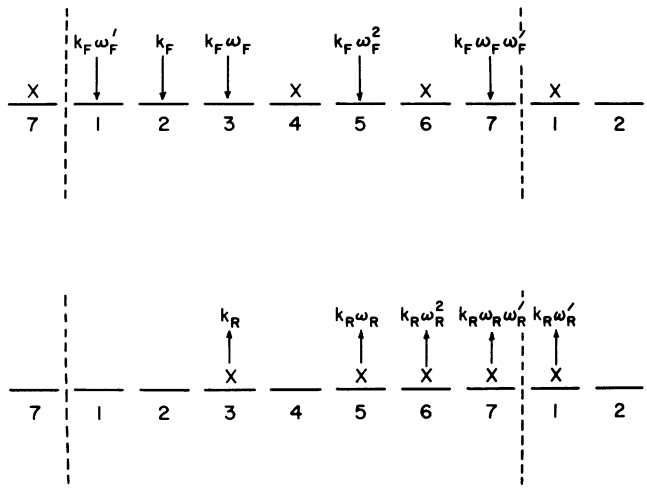

FIGURE 1 Associative and dissociative rate constants arising from the different types of possible events. The numbers under the sites specify their location: site 7 is at the end of one unit, while site 1 is the first site of the adjoining unit. Each X represents a bound myosin head.

$$d\theta_z/dt = M_O(1 - \gamma\theta)\,(1 - \theta_z)k_f e_{fz} - \theta_z k_r e_{rz}, \qquad (8b)$$

where M_O is the concentration of free myosin, γ is the ratio of the actin (site) to myosin concentrations (cf. Part I, Eq. 4) and the "effectivity factors" are given by

$$\begin{aligned} e_{fu} = (1/5)\,[&3(1 - \theta_u^2) + 2(1 - \theta_u)\,(1 - \theta_z) \\ &+ 3\{2\}\omega_f(1 - \theta_u)\theta_u + 2\omega_f\theta_u(1 - \theta_z) \\ &+ 2\omega_f(1 - \theta_u)\theta_z + 3\omega_f^2\theta_u^2 + 2\omega_f^2\theta_u\theta_z] \end{aligned} \qquad (9)$$

$$\begin{aligned} e_{ru} = (1/5)\,[&3(1 - \theta_u^2) + 2(1 - \theta_u)\,(1 - \theta_z) \\ &+ 3\{2\}\omega_r(1 - \theta_u)\theta_u + 2\omega_r\theta_u(1 - \theta_z) \\ &+ 2\omega_r(1 - \theta_u)\theta_z + 3\omega_r^2\theta_u^2 + 2\omega_r^2\theta_u\theta_z] \end{aligned} \qquad (10)$$

$$\begin{aligned} e_{fz} = (1/2)[&2(1 - \theta_z)(1 - \theta_u) + 2\omega_f\theta_u(1 - \theta) \\ &+ 2\omega_f'(1 - \theta_u)\theta_z + 2\omega_f\omega_f'\theta_u\theta_z] \end{aligned} \qquad (11)$$

$$\begin{aligned} e_{rz} = (1/2)[&2(1 - \theta_z)(1 - \theta_u) + 2\omega_r\theta_u(1 - \theta_z) \\ &+ 2\omega_r'(1 - \theta_u)\theta_z + 2\omega_r\omega_r'\theta_u\theta_z]. \end{aligned} \qquad (12)$$

The term $7(1 - \theta)^2$ in Eqs. 15 and 18 of I has been replaced by the first two terms in Eqs. 9 and 10 here. The reason for this change is that now sites 3, 4, and 5 are "inner internal sites," i.e., they have two neighbors of class u, while the "outer internal sites" 2 and 6 have one u neighbor and one z neighbor. Similar considerations apply to the other terms in the corresponding equations.

The system of two differential equations (Eq. 8) is easily integrated far more rapidly than and without the occasional numerical problems encountered with the system of sixteen equations proposed earlier (Balazs and Epstein, 1983). It is thus possible to perform calculations for a far wider range of kinetic parameters (k_r, k_f, ω_r, ω_f, ω_r', ω_f'), system compositions (M_o, γ) and initial conditions (θ at t = 0, i.e., the degree of "preloading"). In Fig. 2, we illustrate the agreement that may be obtained between simulations with this model and experimental data.

The fits obtained in Fig. 2 are in excellent agreement with experiment. However, on varying the model parameters one finds that even a complete set of data on various preloadings at a fixed set of conditions (actin and S1 concentrations, pH, temperature, ionic strength) as obtained by Trybus and Taylor (1980) is inadequate to determine the parameters uniquely. For example, if the value of k_r used in calculating Fig. 2 is doubled and that of ω_f is increased from 6 to 10, the calculated curves fit the data at preloadings of 0, 1, 2, 3, 4, 5, and 6 ligands per actin site nearly as well as those generated with the parameters given in the figure caption. Owing to the compensatory nature of changes in the associative and dissociative

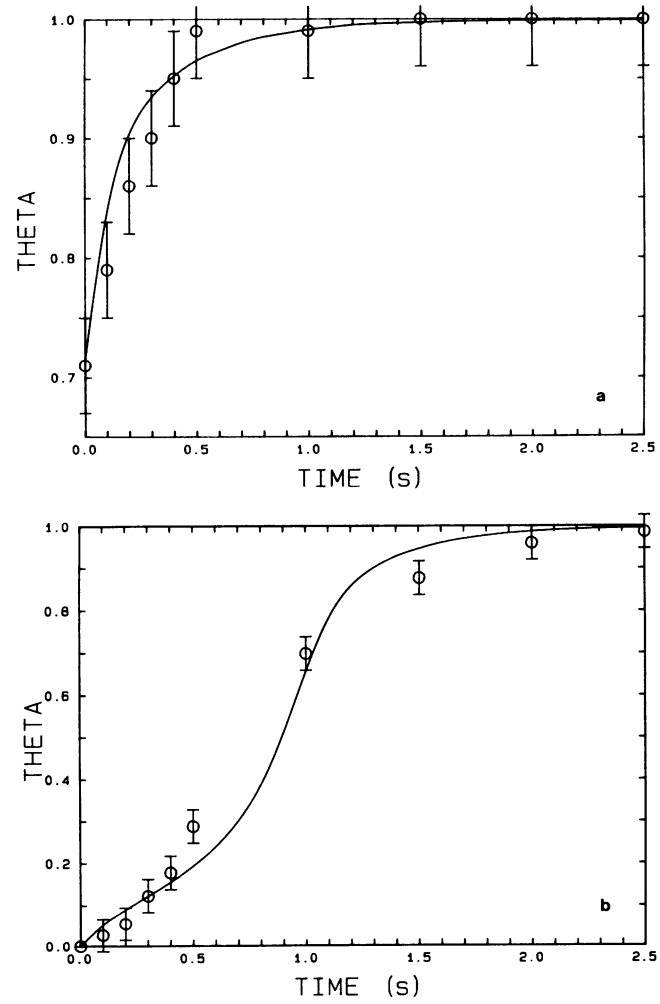

FIGURE 2 Extent of binding of myosin subfragment 1 to regulated actin in the absence of Ca^{2+}. Experimental conditions: 20°C, 10 mM Tris MES (pH 7), 0.2 M KCl, 5 mM $MgCl_2$, 2 mM EGTA, 10^{-6} M actin, 4×10^{-6} M S1. Points: experimental data (Trybus and Taylor, 1980); *solid line*, calculated with simplified two-variable model (Eq. 8) using the following parameters: $k_f = 1.6 \times 10^5\ M^{-1}\ s^{-1}$, $k_r = 10\ s^{-1}$, $\omega_f = 6$, $\omega_r = 0.001$, $\omega_f' = \omega_r' = 1$. (*a*) Initial preloading of 5 S1 monomers per regulated actin unit ($\theta_o = 0.714$); (*b*) no initial preloading ($\theta_o = 0$).

parameters, it is likely that even larger changes could be made in groups of parameters without seriously decreasing the quality of the fit. If data were available over a range of concentrations, it should then be possible to distinguish between alternative sets of model parameters. Unfortunately such data are not yet obtainable.

In the limit that $\omega_f = \omega_f'$ and $\omega_r = \omega_r'$, the model yields the same results as the simpler one-variable system in I with $\theta = \theta_u = \theta_z$. On the other hand, the present model may also be extended to take into account more complex binding phenomena such as the interaction between two-headed myosin molecules and actin filaments.

ACTIN CROSSLINKING AND GELATION

If, instead of S1 subfragments, we consider the binding of the two-headed subfragment HMM (Greene and Eisenberg, 1980), the analysis becomes considerably more complex. Some myosin molecules will bind to actin with both heads, some with only one, and some will remain free. Another type of cooperativity, that between the two heads belonging to a single HMM must be considered. Furthermore, if both heads of a given subfragment bind, they need not bind to the same actin filament, but may instead form a crosslink between two different filaments.

This full array of complexity will be treated elsewhere. For the moment, we focus only on the last problem mentioned, that of crosslinking. We consider the question of whether, for a given composition, a solution consisting of actin and HMM will remain as a sol or will undergo a transition to a gel state. That is, we seek to obtain an expression for the critical sol-gel transition point, θ_{cr}.

To calculate θ_{cr} we utilize the topological gelation criterion (TGC) proposed by Klonowski (1983, 1984, 1985). The TGC is discussed in detail by Klonowski (1985) where its relationship with classical models of gelation (Flory, 1953) is treated. For simplicity we assume that all actin chains are identical, each having X binding sites. If necessary, an averaging over the molecular weight distribution and hence over the distribution of X may be performed (Klonowski, 1979).

Consider a system consisting of two kinds of macromolecules, N_A molecules of type A, each bearing X_A reactive groups, and N_B molecules of type B, each with N_B reactive groups, such that one A group may form a crosslink by reacting with one B group (cf. discussion below). A special case of the TGC yields an algebraic equation for the critical extent $\alpha = \alpha_{cr}$ of the crosslinking reaction (Klonowski, 1985, Eq. 37 for $\beta = 0$ and Table I)

$$\begin{aligned}&\{2 - 2(1 - \alpha^A)^{X^A-1}[(X^A - 1)\alpha^A + 1]\}/X^A\alpha^A \\ &+ \{2 - 2(1 - \alpha^B)^{X^B-1}[(X^B - 1)\alpha^B + 1]\}/X^B\alpha^B \\ &= 2[1 - (1 - \alpha^A)^{X^A-1} - (1 - \alpha^B)^{X^B-1} \\ &+ (1 - \alpha^A)^{X^A-1}(1 - \alpha^B)^{X^B-1}],\end{aligned} \tag{17}$$

where α^A and α^B are expressed in terms of the global extent of reaction α and stoichiometric ratio (Klonowski, 1984)

$$\gamma = A_o/B_o = N_A X^A/N_B X^B \tag{18}$$

as

$$\alpha^A = (\alpha/2) \cdot (1 + 1/\gamma) \tag{19a}$$

$$\alpha^B = (\alpha/2) \cdot (1 + \gamma). \tag{19b}$$

In the present case we let A represent actin and B myosin (HMM). The extent of reaction of actin is then identical with θ:

$$\alpha^A = \theta \tag{20}$$

and

$$X^A = X \tag{21}$$

$$X^B = 2. \tag{22}$$

Using Eq. 20, Eq. 19b now becomes

$$\alpha^B = \gamma\theta \tag{23}$$

and Eq. 17 can now be simplified to

$$2(1 - \theta)^{X-1}[(1 - \theta) + X\theta(1 - \gamma\theta)] + \gamma X\theta^2 - 2 = 0. \tag{24}$$

Eq. 24 is easily solved numerically to yield the critical sol-gel transition point for the system under consideration at any given values of X and γ. Typical results are given in Table I. For $X \geq 7$, the first term $(1 - \theta)^{X-1}$ is quite small and we obtain

$$\theta_{cr} \approx (2/\gamma X)^{1/2}. \tag{25}$$

Values of θ_{cr} obtained with the approximate Eq. 25 are ~5% smaller than those from the exact Eq. 24 at $X = 7$ and only about 0.001% smaller even at $X = 70$ and $\gamma = 0.1$.

TABLE I
CRITICAL SOL-GEL TRANSITION POINT, θ_{cr}, FOR ACTIN-HMM SYSTEMS (FROM EQ. 24)

r^* | γ	0.1	0.2	0.5	1.0	2.0	5.0	10.0
1	—	—	0.7556	0.5279	0.3601	—	—
2	—	0.8452	0.5345	0.3764	0.2606	0.1563	—
3	0.9759	0.6901	0.4364	0.3081	0.2153	0.1300	0.0875
4	0.8452	0.5976	0.3780	0.2671	0.1876	0.1143	0.0775
5	0.7556	0.5345	0.3380	0.2390	0.1683	0.1034	0.0701
6	0.6901	0.4880	0.3086	0.2182	0.1539	0.0951	0.0647
7	0.6389	0.4518	0.2857	0.2020	0.1426	0.0886	0.0604
8	0.5976	0.4226	0.2672	0.1890	0.1335	0.0832	0.0569
9	0.5634	0.3984	0.2520	0.1782	0.1259	0.0787	0.0540
10	0.5346	0.3780	0.2390	0.1690	0.1120	0.0756	0.0515
10^2	0.1690	0.1195	0.0756	0.0535	0.0378	0.0240	0.0169
10^3	0.0535	0.0388	0.0240	0.0169	0.0120	0.0076	0.0053
10^4	0.0169	0.0120	0.0076	0.0053	0.0038	0.0024	0.0017

*$r = X/7$ is the number of regulated actin units.

From the definition of an extent of reaction it is obvious that α, α^A, and α^B (Eqs. 19a and b) must all be positive and less than or equal to unity. Using Eqs. 20 and 23 we may then obtain a necessary condition on the parameters X and γ in order for gelation to take place

$$\frac{2}{X} \leq \gamma \leq \frac{X}{2}. \tag{26}$$

If the left inequality is not fulfilled, the concentration of actin is too small; if the right inequality is not satisfied, the concentration of myosin is too small for gelation.

The results obtained in Eqs. 24 and 26 and in Table I provide a potential test of the model proposed and of the TGC. The hydrostatic device described by Stossel (1983) for determining the critical gel point of actin-binding protein (Hartwig et al., 1977) could be utilized to study the behavior of actin-HMM preparations as a function of the molecular weight distribution of the actin and the results compared with the above predictions.

It should be noted that in earlier papers (Klonowski 1979, 1983, 1984, 1985) in which the theory leading to Eq. 7 is developed, a crosslink is defined as above, i.e., as a single link between an A group and a B group. Such a definition is clearly appropriate when both A and B are long chain macromolecules, and we have maintained that convention for consistency with the previous work. In the present case, however, a more natural way to define a crosslink would be as one HMM molecule, B, with both its binding sites linked to actin molecules, A. In terms of the parameters defined above, the concentration of such myosin bridges is given by

$$B = N_B(\alpha^B)^2 = \frac{M_o\gamma^2\theta^2}{2}. \tag{27}$$

DISCUSSION

The previous section dealt with HMM crosslinking only in regard to the question of sol-gel transition. By introducing additional equations and parameters that account for the new modes of binding and cooperativity that become available, one can extend Eq. 8 into a general model for the kinetics of binding mixtures of S1 and HMM to regulated actin. A six-variable version of such a model has been constructed, and details will be published elsewhere. Greene and Eisenberg (1980) have studied experimentally the equilibrium binding of S1-HMM mixtures to F-actin.

It is well known that positive feedback and autocatalysis, of which inter-site cooperativity may be considered an example, can give rise to chemical oscillation. Schneider and Rawlings (1971) have carried out a prototype calculation for the case of helix-coil transition. If for some set of parameters or experimental conditions, the number of crosslinks in an actin-myosin system oscillates about the critical value θ_{cr} given by Eq. 24, then a new type of dissipative structure (Nicolis and Prigogine, 1977). a sol-gel dissipative structure, will be generated. The system will spend part of each cycle in a liquid-like sol state, undergo an abrupt transition to the solid-like gel state, then return to the sol state, etc. The system will display a periodic pattern in time (Klonowski, 1985). If diffusion of the crosslinking agent (HMM) plays a significant role, then spatial or spatio-temporal sol-gel patterns may appear.

The model described above is still too simple to produce such phenomena. In addition to containing an appropriate feedback, the system must also be open in the thermodynamic sense. Regulating species that produce the feedback should probably be considered. A full model would take into account the role of ATP in the breaking of acto-myosin linkages and the dependence of reaction rates on ATPase activity. The constant influx of ATP serves to keep the system open and far from equilibrium, thus making oscillations possible. Another key factor may be the influence of proteins that regulate the molecular weight distribution of the actin chains.

Further work on the possibility of sol-gel dissipative structures in actin–myosin preparations is now in progress. It is of interest that Kaufman et al., (1986) have recently observed temporal oscillations in doped silica that are apparently due to just this sort of repeated sol-gel transition.

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